CHRONIC TOXICITY SUMMARY

CHLORINE

CAS Registry Number: 7782-50-5

I. Chronic Toxicity Summary

Inhalation reference exposure level	**0.2 µg/m^3 (0.08 ppb)**
Critical effect(s)	Hyperplasia in respiratory epithelium in female rats
Hazard index target(s)	Respiratory system

II. Physical and Chemical Properties (HSDB, 1995; 1999; CRC, 1994)

Description	Yellow/green gas
Molecular formula	Cl_2
Molecular weight	70.905
Density	2.9 g/L @ 25°C and 1 ATM
Boiling point	-34.6°C
Melting point	-100.98°C
Vapor pressure	5 atm @ 10.3°C; 5830 torr @ 25°C
Solubility	Slightly soluble in water (310 mL per 100 mL water at 10° C; 1.46 g per 100 mL water at 0° C)
Conversion factor	1 ppm = 2.9 mg/m^3 @ 25° C

III. Major Uses and Sources

In an industrial setting, chlorine is widely used as an oxidizing agent in water treatment and chemical processes. Chlorine is also used to disinfect swimming pool water. Chlorine gas is sometimes used at large public pools while household pools typically use hypochlorite solutions. Chlorine is an integral part of the bleaching process of wood pulp in pulpmills, although chlorine dioxide is replacing this use of chlorine. Chlorine as sodium hypochlorite is commonly used as a household cleaner and disinfectant (HSDB, 1995). The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 244,955 pounds of chlorine (CARB, 1999).

IV. Effects of Human Exposure

Shi and associates (1990) evaluated 353 workers from a diaphragm cell chlorine chemical plant. The workers ranged in age from 23-52 years with an average of 42.4 years. Two groups were compiled with respect to the workers' length of exposure in years. Group A consisted of 220

workers who were employed/ exposed for 10-25 years. Group B consisted of 133 workers employed for less than 10 years. Both groups of workers were exposed to a range of 2.60-11.0 mg/m^3 (0.37-1.75 ppm) chlorine. The control group's average age was 39.7 years (ranging from 26-55 years), and it consisted of 192 workers not exposed to chlorine, but working within the same plant. For all the groups, respiratory symptoms and smoking habits were evaluated as well as clinical examinations, ENT examinations, chest x-rays and pulmonary function tests. Groups A and B showed 3-8 times higher incidence of upper airway complaints than the control workers. Current smokers in groups A and B experienced the highest incidence of pulmonary symptoms and group A workers had a higher prevalence of rhino-pharyngeal signs than the control workers. Abnormalities in chest x-rays were seen in 8.6% of group A workers and in 2.8% of group B workers, compared to 2.3% of the control workers. Groups A and B showed significantly impaired pulmonary function in tests of V50/H and FEF_{25-75} (forced expiratory flow between 25 and 75% of forced vital capacity (FVC), the total amount of air the subject can expel during a forced expiration) - compared with the control group, and group A showed reduced FEV_1 (forced expiratory volume in 1 second) results compared to the control group.

Kennedy *et al.* (1991) compared 321 pulpmill workers (189 of whom were exposed to chlorine or chlorine dioxide "gassings") to a control group of 237 rail yard workers in similar working conditions but not exposed to chlorine (79% and 84% respective participation rates). The workers had been employed for an average of 13 years at the pulpmill and 12.7 years at the rail yard. Chlorine gas and chlorine dioxide levels were measured together over a 4 week period during mainly a 12 hour shift. Time weighted averages (TWA) were <0.1 ppm, with the highest of <0.1-0.3 ppm. A significantly higher prevalence of wheezing was seen in pulpmill workers (both smokers and nonsmokers) who had reported more than one episode of chlorine "gassing" as compared to the rail yard workers and pulpmill workers with no chlorine gas exposure. More airflow obstruction was observed in exposed workers in spite of their nonsmoking and ex-smoking status, correlating to significantly lower average values for MMF (maximal mid-expiratory flow) and for the FEV_1 to FVC ratio. Comparison of pulpmill workers exposed to chlorine and /or chlorine dioxide with those pulpmill workers not exposed, suggests that chronic respiratory health impairment is associated with exposure to chlorine and/or chlorine dioxide. These researchers hypothesized that after the first high exposure incident, an inflammatory response occurred in small airways and that this reaction did not resolve in those workers who were continuously or repeatedly exposed to the irritant. It was also suggested that chronic airflow obstruction caused by repeated minor exposures led to chronic respiratory disability in some of the workers.

Patil *et al.* (1970) evaluated the exposure of 332 male diaphragm cell workers to 0.006-1.42 ppm chlorine gas (a range with a time-weighted average of 0.146 $\pm$0.287; most workers were exposed to less than 1 ppm). A control group consisting of 382 workers from 25 representative chlorine manufacturing plants was also studied. Both groups were comprised of men between the ages of 19-69 with a mean age of 31.2 $\pm$11.0 years. Physical examinations (blood and urine analysis, chest x-rays and electrocardiograms) were conducted, in most cases, within the first six months of the study year. At two month intervals, each plant was surveyed and chlorine levels were determined. Exposed employees were grouped according to job classification. Researchers found the average number of exposure years for the study group to be 10.9 $\pm$ 2.8 years and concluded that the exposure level had no correlation to the number of years exposure. Ninety-

eight of the 332 workers were found to have abnormal teeth and gums, but no dose-response relationship was concluded. Similarly, no dose-response relationships were shown with the symptoms of sputum production, cough, dyspnea, history of frequent colds, palpitation, chest pain, vital capacity, maximum breathing capacity and forced expiratory volume. Any deterioration in pulmonary function was shown to be age related. Of the 332 exposed workers, 9.4% experienced abnormal EKGs. 8.5% of the control group showed the same abnormalities, but this difference was not significant. Above 0.5 ppm, an increase appeared in the incidence of fatigue. No neurological defects developed and there was no noted prolonged anoxia as a result of the chlorine exposure. Also, no consistent gastrointestinal trouble or abnormal incidence of dermatitis was found. Exposed workers showed elevated white blood cell counts and decreased hematocrit values compared to the control group.

Bherer *et al.* (1994) conducted a follow up study of the Quebec pulp mill research done by Courteau and associates over a time interval of 18-24 months after the incidents of repeated exposures. Fifty-eight of the original 289 exposed workers from the moderate to high risk group were studied for developing reactive airways dysfunction syndrome (RADS). Workers at a moderate risk were defined as having shortness of breath after their most significant exposure, but not at the time of the initial study by Courteau *et al.* Moderate risk workers also had a record of other significant medical conditions and/or were 50 years of age or older. High risk workers were defined as those experiencing shortness of breath that continued one month after the exposure and/or abnormal lung sounds. Ninety percent of the follow up group completed questionnaires which revealed a 91% incidence of respiratory symptoms. Spirometry assessments and methacholine inhalation tests were conducted on 51 of the 58 workers. Twenty-three percent of the 58 workers still experienced bronchial obstruction and 41% continued to have bronchial hyper-responsiveness. Lower baseline FEV_1 was seen in those with a lower PC_{20}, and 52% of these workers showed an $FEV_1 < 80\%$ predicted.

Enarson *et al.* (1984) compared 392 pulpmill workers exposed to chlorine (unspecified duration) to a comparable group of 310 rail yard workers living in the same community, but not exposed to chlorine. In the pulpmill areas surveyed that predominantly had significant chlorine gas levels (machine room and bleach plant), workers were exposed to either an average of 0.02 ppm or 0.18 ppm Cl_2 respectively. Of the machine room workers, 23.2% experienced a cough as did 32.8% of those in the bleach plant, compared to 22.3% of the control rail yard workers. Chest tightness occurred in 31.5% of the machine room workers and 39.6% of the bleach plant workers as compared to 21.3% of the control. Only data from Caucasian subjects were reported.

Chester *et al.* (1969) evaluated 139 workers occupationally exposed to <1 ppm chlorine for an unspecified duration. Fifty-five of the 139 workers were exposed to additional accidental high concentrations of chlorine, which were severe enough to require oxygen therapy. Ventilation was affected by chlorine inhalation, with a decrease in the maximal midexpiratory flow (MMF). Smokers in this group had significantly reduced FVC, FEV_1 and MMF compared to nonsmokers. Fifty-six of the 139 subjects showed abnormal posteroanterior chest films, 49 of which had parenchyma and/or hilar calcifications consistent with old granulomatous disease and 11 of which had multiple, bilateral and diffuse calcifications. Researchers suggest that the first ventilation function affected in obstructive airway disease is MMF.

V. Effects of Exposure to Animals

Wolf *et al.* (1995) exposed male and female B6C3F1 mice and F344 rats to chlorine gas concentrations of 0 ppm, 0.4 ppm, 1.0 ppm and 2.5 ppm. The exposures were carried out for 104 weeks at 6 hr/day 3 days/week for female rats and 6 hr/day 5 days/week for mice and male rats. Based on previous studies, the authors determined that female rats could not tolerate 5 days/week exposure to chlorine. Each treatment group contained 320 male and 320 female mice. The rats were studied in groups of 70, yielding 280 per gender per species. For the first 13 weeks of observation, body weights and clinical observations were noted weekly, and for the remainder of the study, they were recorded once every two weeks. After 52 weeks, 10 rats were euthanized and autopsied. Organ weights were recorded, and hematologic and clinical chemistry parameters were determined. These same measurements were performed on all of the surviving mice and rats at the conclusion of the 104 weeks. Male mice exposed to 1.0 and 2.5 ppm Cl_2 showed decreased weight gain compared to controls while only female mice exposed to 2.5 ppm Cl_2 showed decreased weight gain. Male rats showed decreased weight gain at all levels of exposure while female rats showed the same result at only 1.0 and 2.5 ppm Cl_2 exposures. Various nonneoplastic nasal lesions were seen in all the airway epithelial types in the nose and at all levels of exposures for both species. These lesions were evaluated against background lesions found in the control animals. A statistically significant incidence of fenestration was seen in all three exposure concentrations of Cl_2. Statistically significant responses were seen in the traditional and respiratory epithelial regions of all exposed rats and mice. Statistically significant damage to olfactory epithelium occurred in all exposed rats and female mice and also in the 1.0 and 2.5 ppm exposed groups of male mice.

Klonne *et al.* (1987) exposed 32 male and female rhesus monkeys to chlorine gas for one year to measured concentrations of 0, 0.1, 0.5, and 2.3 ppm Cl_2. These monkeys were exposed to chlorine for 6 hours/day, 5 days/week. The monkeys were evaluated periodically on the basis of body weight, electrocardiograms, neurologic examinations, pulmonary function, hematologic parameters, serum chemistry, urinalysis, and blood gas and pH levels. Results were compared to the same test measurements recorded prior to the study. No significant difference was seen in body weight at any point in the experiment. Ocular irritation (tearing, rubbing of the eyes, reddened eyes) was observed after 6 weeks of exposure in the 2.3 ppm group. No exposure-related differences were seen in neurologic examinations, electrocardiograms, clinical chemistry, urinalysis, hematology or blood gas levels. Also, no exposure-related changes were observed in the parameters of ventilation distribution. Pulmonary function evaluations yielded a statistically significant trend for increasing pulmonary diffusing capacity and distribution of ventilation values for males and females in the 2.3 ppm exposure group. Both males and females of the 2.3 ppm group exhibited statistically significant increased incidence of respiratory epithelial hyperplasia. A mild form of the lesions was also seen in the 0.5 ppm group, 0.1 ppm group (females only) and one male in the control group. Two parasitic infections occurred, affecting the respiratory tract and resulting in 11 monkeys housing parasites and/or ova. Additionally, 16 monkeys displayed histologic changes characteristic of the presence of the parasites. However, the parasitic induced lesions were not associated with lesions in the respiratory epithelium.

VI. Derivation of Chronic Reference Exposure Level (REL)

Study	Wolf *et al.*, 1995
Study population	Female F344 rats (70 per group)
Exposure method	Discontinuous whole-body inhalation exposure (0, 0.4, 1.0 or 2.5 ppm)
Critical effects	Upper respiratory epithelial lesions (see following table)
LOAEL	0.4 ppm
NOAEL	Not established
BMC_{05}	0.14 ppm
Exposure continuity	6 hours/day, 3 days/week (MWF)
Average experimental exposure	0.015 ppm
Human equivalent concentration	0.0024 ppm (gas with extrathoracic respiratory effects, RGDR = 0.16 based on BW = 229 g, MV = 0.17 L/min, SA(ET) = 15 cm^2)
Exposure duration	2 years
LOAEL uncertainty factor	(not needed in the BMC approach)
Subchronic uncertainty factor	1
Interspecies uncertainty factor	3
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	30
Inhalation reference exposure level	0.08 ppb (0.20 µg/m^3)

A benchmark dose analysis was performed using a log-normal probit analysis (Tox-Risk, version 3.5; ICF-Kaiser Inc., Ruston, LA) of the female rat data. Using the data for glandular epithelial eosinophilic proteinaceous accumulation (see Table 1 below) to derive the BMC_{05} resulted in a 3-fold lower value than the LOAEL of 0.4 ppm, or BMC_{05} = 0.14 ppm. (Adequate benchmark dose estimates could not be obtained for the other nasal lesions due to high background rates and shallow dose-response relationships.) A BMC_{05} is considered to be similar to a NOAEL in estimating a concentration associated with a low level of risk.

The Wolf *et al.* (1995) study of mice and rats was chosen as the key reference for the chlorine chronic REL for several reasons. First, the duration of the experiment was for a full lifetime of two years. Second, the sample sizes were large (280 per sex per species). Finally, appropriate sensitive endpoints of respiratory epithelial damage were examined. The mice and male rats were exposed to chlorine for 6 hours/day, 5 days/week, but the female rats were only exposed for 3 days/week as the authors observed the females to be more sensitive than the males. Table 1 shows the histological findings of the female rats. Statistically significant results ($p < 0.05$) were seen for all the tissues at 0.4 ppm chlorine exposure and above.

Table 1. Female Rat Epithelial Lesions following Chronic Chlorine Exposure (based on Table 5 of Wolf *et al.*, 1995)

Tissues	0 ppm	0.4 ppm	1.0 ppm	2.5 ppm
Goblet cell hyperplasia	3/70 (4%)	50/70 (71%)	63/70 (90%)	64/70 (91%)
Respiratory epithelium eosinophilic proteinaceous accumulation	49/70 (70%)	60/70 (85%)	59/70 (84%)	65/70 (93%)
Glandular epithelium eosinophilic proteinaceous accumulation	**16/70 (23%)**	**28/70 (40%)**	**52/70 (75%)**	**53/70 (76%)**
Olfactory epithelium eosinophilic proteinaceous accumulation	36/70 (52%)	64/70 (91%)	69/70 (99%)	69/70 (99%)

The Wolf *et al.* (1995) study was chosen over the Klonne *et al.* (1987) monkey study for the following reasons: the monkeys were exposed for only one year of their total 35 year lifetime, and the sample sizes were considerably smaller (4 monkeys per sex per group) than the mouse and rat groups (280 per sex per species). Although the exposure durations differed between the two studies, the histological results were similar, differing only slightly in the region of occurrence. The monkeys displayed both tracheal and nasal lesions. Both the rodents and the monkeys showed upper respiratory epithelial lesions, thus suggesting that the rodents may be an appropriate model for humans.

For comparison with the proposed REL of 0.08 ppb (0.2 $\mu g/m^3$) using the BMC approach, we estimated a REL of 0.02 ppb (0.06 $\mu g/m^3$) based on the same rat study but using the NOAEL/UF approach with a LOAEL of 0.4 ppm divided by a total UF of 300 (10 for LOAEL, 3 for interspecies, and 10 for intraspecies) and the RGDR of 0.16. As another comparison, using 0.1 ppm as a LOAEL for respiratory epithelial lesions in female monkeys, the LOAEL can be time-adjusted to an equivalent continuous value of 24 ppb. Applying a UF_L of 3 for a mild effect, a UF_S of 10 since it was only a 6 month study, an interspecies UF of 3 for monkeys, and an intraspecies UF of 10 results in an estimated REL of 0.02 ppb (0.06 $\mu g/m^3$).

The human studies were examined for possible use in the calculation of a REL. The studies were limited by very variable exposures (e.g., Patil *et al*. (1970)), the presence of serious adverse health effects in some workers (chest x-ray abnormalities in Shi (1990), abnormal teeth and gums in 98 of 332 workers in Paril *et al*. 1970)), exposure to other compounds such as chlorine dioxide (Kennedy *et al*. (1991)), multiple acute "gassings" with chlorine (Kennedy *et al*. (1991)), and absence of data on cigarette smoking, also a respiratory system irritant. As an illustration of what would be estimated, the study of Shi (1990) had a mean workplace exposure of 4.82 mg/m^3 (1.7 ppm). This LOAEL was time adjusted to an equivalent continuous exposure of 1.72 mg/m^3,

then divided by an uncertainty factor of 100 (10 for use of a LOAEL and 10 for intraspecies variability) to yield a REL of 20 $\mu g/m^3$ (7 ppb). However, the use of a LOAEL default uncertainty factor of 10 does not seem adequate for frank, possibly irreversible effects such as the chest x-ray abnormalities reported. There is currently no methodology to deal with such effects in REL development.

Adequate benchmark dose estimates could not be obtained for the other nasal lesions due to high background rates and shallow dose-response relationships.

VII. Data Strengths and Limitations for Development of the REL

The strengths of the inhalation REL for chlorine include the availability of chronic multiple-dose inhalation exposure data from a recent (1995), well-conducted animal study with histopathological analysis. Major areas of uncertainty are the lack of adequate human exposure data, the lack of observation of a NOAEL, and limited reproductive toxicity data.

VIII. References

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